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Estrogen Replacement in a Protein S Deficient Patient Leads to Diarrhea, Hyperglucagonemia, and Osteonecrosis

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ABSTRACT

Context Protein S deficiency and mesenteric venous thrombosis have been described in association with ischemic and/or necrotic bowel. Thrombophilic familial protein S deficiency is known to be amplified by estrogen replacement therapy. Pancreatic ischemia studies have revealed elevated amylase and lipase levels but not hyperglucagonemia. We postulate that estrogen replacement therapy leading to mesenteric and pancreatic ischemia not only caused symptoms of ischemic bowel, but also pancreatic oversecretion of glucagon in a patient with protein S deficiency. Our specific aim was to assess thrombophilic interactions of estrogen replacement therapy and familial protein S deficiency leading to osteonecrosis, hyperglucagonemia, and diarrhea.

Case Report Premarin (2.5 mg/day) was begun following bilateral oophrectomy at age 37. At age 56, hip replacement was done for osteonecrosis of the femoral head. Subsequently, severe epigastric pain and diarrhea developed, which persisted despite conservative measures. Diagnostic evaluation revealed hyperglucagonemia (1420 pg/mL). Although abdominal sonograms, CT scans, and endoscopy failed to document a glucagon-secreting tumor, octreotide (50 μg/day) was begun. Normalization of glucagon levels and improvement of abdominal pain was achieved; diarrhea (5-6 episodes/day) persisted.

Serologic and genetic testing revealed thrombophilic familial protein S. After stopping estrogen replacement therapy and octreotide, diarrhea and abdominal pain disappeared, glucagon remained normal (normal after 30 months follow-up), and free and functional protein S remained low.

Conclusions Estrogen induced reduction of protein S, superimposed on familial protein S deficiency, led to osteonecrosis and then, speculatively, to thrombotic mesenteric and pancreatic ischemia with resultant diarrhea, abdominal pain, and hyperglucagonemia. Diarrhea, abdominal pain, and hyperglucagonemia normalized when estrogen was discontinued, and have remained normal over 30 months follow-up.

INTRODUCTION

Hyperglucagonemia is usually caused by excessive production of glucagon from glucagon-secreting tumors of the pancreas [1, 2, 3, 4, 5, 6]. However, hyperglucagonemia can occur in diverse conditions such as chronic renal insufficiency, diabetic ketoacidosis, prolonged starvation, acute pancreatitis, acromegaly, hypercorticism, septicemia, severe stress, familial hyperglucagonemia, and hepatic insufficiency [1, 2, 3, 4, 5, 6]. Manifestations of hyperglucagonemia include dermatitis, diabetes, diarrhea, weight loss, abdominal pain, psychiatric illness, and thromboembolic disease [1, 2, 3, 4, 5, 6]. In cases of hyperglucagonemia caused by a glucagonoma, the tumor localized to the pancreas can usually be identified by means

of a CT scan, or by direct sonography of the pancreas [1, 2, 3, 4, 5, 6].

Protein S is a vitamin K dependent plasma protein synthesized by the liver. In combination with protein C, its function is to inactivate factors Va and VIIIa of the coagulation cascade, thus regulating thrombus formation [7, 8]. Protein S deficiency can lead to thrombotic arterial and venous occlusive events [9] including ischemic stroke [10, 11], deep vein thrombosis [12], and thromboembolism. Familial protein S deficiency has been shown to cause osteonecrosis of the hip, probably by promoting formation of venous thrombi in the major veins which drain the head of the femur [13]. The resultant increased bone venous pressure and reduction of arterial flow apparently produces bone hypoxia and leads to bone death [13].

Exogenous estrogen can be a cofactor in thromboembolism, augmenting the thrombotic complications of protein S deficiency [14, 15, 16]. Estrogen replacement therapy (ERT) can reduce antigenic, functional, and free protein S levels, thus promoting thrombophilia [14, 15, 16]. Estrogen-induced lowering of protein S [14, 15, 16], when superimposed on familial protein S deficiency [14], amplifies thrombophilia. This is similar to estrogen-induced resistance to activated protein C which, when superimposed on the Factor V Leiden mutation, further increases resistance to activated protein C [17, 18, 19]. In such cases, the risk of venous thrombosis is increased by 80-100 fold [17, 18] and the risk of arterial thrombosis by 2 fold [19].

Thrombophilia and hypofibrinolysis have been shown to be major pathoetiologies for osteonecrosis [13, 17, 18, 20, 21, 22, 23]. Major heritable thrombophilic traits associated with osteonecrosis include protein C and/or S deficiency, the Factor V Leiden mutation, and hyperhomocysteinemia (homozygosity for the methylenetetra-hydrofolate reductase (MTHFR) polymorphism) [13, 17, 18, 20, 21, 22, 23]. Additional heritable/acquired thrombophilias which appear to cause osteonecrosis include anticardiolipin antibodies and the lupus anticoagulant [20, 21, 22, 23]. Major familial hypofibrinolytic traits associated with osteonecrosis include homozygosity for the 4G4G polymorphism of the plasminogen activator inhibitor-1 (PAI-1) gene with attendant high plasminogen activator inhibitor activity (PAI-Fx), and lipoprotein (a) (Lp(a)) [20, 21, 23].

In the present study, our specific aim was to describe the thrombophilic interaction between ERT and protein S deficiency in a patient with familial protein S deficiency leading to osteonecrosis [13] of the hip, "idiopathic" hyperglucagonemia, intractable diarrhea, and abdominal pain.

METHODS

After an overnight fast, blood samples were obtained at 8:30 am in the seated position. Serologic and PCR measures were made of thrombophilia and hypofibrinolysis using previously published methods [13, 17, 19, 21, 23, 24, 25]. Serologic tests of thrombophilia included total, free, and functional protein S, protein C, antithrombin III, anticardiolipin antibodies IgG and IgM, the lupus anticoagulant, and resistance to activated protein C. Serologic tests of hypofibrinolysis included Lp(a) and plasminogen activator inhibitor activity. PCR tests for thrombophilia included factor V Leiden, MTHFR, prothrombin, and platelet glycoprotein IIIa gene PL A1/A2 polymorphisms. PCR tests for hypofibrinolysis included evaluation of the 4G5G polymorphism of the PAI-1 gene.

The patient has had follow-up for 30 months after her initial evaluation.

ETHICS

The study followed protocols approved by the Jewish Hospital institutional review board, with signed informed consent.

CASE REPORT

The 57-yr-old female proband had a significant past medical history with complete

hysterectomy at age 37 following heavy uterine bleeding that was not controlled by oral contraceptives. ERT (Premarin 2.5 mg daily) was started at age 37 for treatment of perimenopausal symptoms and vaginal epithelial atrophy. At age 55, two years before our study, the patient developed left hip pain, which was diagnosed by X-ray and MRI examination to be osteonecrosis [13]. Secondary causes of osteonecrosis including long term and/or high dose corticosteroids, alcoholism, trauma, sickle cell disease, systemic lupus erythematosus, or Gaucher's disease were not present [20, 21, 22, 23]. Segmental collapse of the head of the left femur necessitated total left hip replacement which was performed following failure of core decompression.

At age 55.5, the patient developed intractable diarrhea, severe abdominal pain, bloating, depression, and generalized weakness. An upper endoscopy (EGD) was performed and revealed only diffuse gastritis. The *Helicobacter pylori* test was negative. Omeprazole was started as treatment for gastritis. Symptomatic treatment for the patient's diarrhea was entirely ineffective; ova, parasite, stool studies, and cultures were negative. This prompted a more extensive evaluation including a chest X-ray, an abdominal and pelvic CT, and an exhaustive battery of laboratory studies (serum electrolytes, complete blood cell count, liver enzymes, glucagon, amylase, lipase, erythrocyte sedimentation rate, rheumatoid factor, anti-nuclear antibodies, urinalysis, and vasoactive intestinal peptide). The only abnormality found was a markedly elevated glucagon level of 1420 pg/mL (reference range: 25-250 pg/mL), congruent with her major symptoms of intractable diarrhea. None of the other laboratory tests or imaging studies were abnormal.

The focus of the investigation thus became to identify the source of the elevated glucagon with the presumption that a tumor had been missed by the initial imaging studies. Imaging with Indium III labeled octreotide showed no abnormal accumulation of radionuclide. Additional diagnostic studies included a dual phase pancreatic CT which showed a normal pancreas in both arterial and venous phases. Exocrine pancreatic function was normal (normal secretin-cholecystokinin test, normal intraluminal digestion products). An EGD with ultrasound was performed demonstrating resolution of the previously diagnosed diffuse gastritis; it failed to provide evidence of tumor in the pancreas. A colonoscopy was unremarkable and biopsies of the rectum and colon showed no evidence of collagenous or microscopic colitis.

Due to the failure of the above studies to localize a tumor, the patient's attending physicians held discussions concerning the possibility that the glucagon was of exogenous origin and was being covertly administered by the patient herself. This suspicion could not be documented, however, and the physicians sought to relieve the patient's unrelenting symptoms through the use of daily octreotide (50 μg subcutaneously). Octreotide therapy was mostly successful, normalizing glucagon levels and ameliorating the symptoms of abdominal bloating and lethargy. Unfortunately, the patient's diarrhea persisted unabated. In pursuit of all possible causes of the patient's osteonecrosis and persistent diarrhea (5-6 episodes of non-bloody diarrhea daily), she was referred at age 57 to the Jewish Hospital Cholesterol Center.

The patient's family history was notable for a predisposition to major thrombotic events. Her father had multiple episodes of lower extremity deep vein thrombosis and multiple, ultimately fatal ischemic strokes. Her mother died at age 52, also from an ischemic stroke. Studies for both familial and acquired causes of thrombophilia and hypofibrinolysis were begun at the Cholesterol Center. The following thrombophilic traits were found. Free protein S was very low (48%; lower normal limit: 65%) and remained low despite cessation of estrogen replacement therapy. The patient was also found to be heterozygous for the thrombophilic PL A1/A2 polymorphism of the platelet glycoprotein IIIa gene. All other causes of thrombophilia were normal including wild-type normal Factor V,

normal MTHFR, and normal prothrombin genes. Studies of heritable hypofibrinolysis revealed elevated Lp(a) (71 mg/dL; upper limit normal limit: 35 mg/dL). There were no other causes of hypofibrinolysis. Family screening revealed protein S deficiency in the patient's 37 year old daughter (free protein S: 30%; lower normal limit: 65%).

To avoid further venous or arterial thrombi related to the thrombophilic interaction of ERT with protein S deficiency [14, 15, 16, 17, 18, 19], and because the patient wished to stop the octreotide, ERT and octreotide were discontinued after the initial visit. At follow-up visits, the patient's free and functional protein S remained very low (<50%) while glucagon remained normal despite cessation of octreotide. After 30 months follow-up, glucagon remains normal, 50 pg/mL. The patient's diarrhea and severe abdominal pain, which had continued despite the octreotide therapy, disappeared, and have not recurred after 30 months follow-up.

DISCUSSION

Given the patient's family history of thrombosis and her previously unexplained "idiopathic" osteonecrosis, we postulated that her osteonecrosis, hyperglucagonemia, diarrhea, and abdominal pain were caused by a familial coagulation disorder, probably amplified by ERT mediated thrombophilia [17, 18, 19]. This postulate was duly realized; she was found to have familial thrombophilia (protein S deficiency, PL A1/A2 polymorphism of the platelet glycoprotein IIIa gene) and hypofibrinolysis (high Lp(a)). What remained uncertain was how the coagulation disorder related to the gastrointestinal symptoms and to the hyperglucagonemia. After simultaneously stopping ERT and octreotide therapy, which (octreotide) had successfully normalized and maintained her glucagon levels, we expected her glucagon levels to quickly rise. We were surprised to find, however, that glucagon levels remained entirely normal and are normal after 30-month follow-up. An equally surprising development after stopping ERT was the rapid, complete resolution of the patient's chronic diarrhea and abdominal pain, which had been unresolved on octreotide. These observations, along with the failure of the diagnostic imaging to localize a glucagon-secreting tumor, led to the following hypothesis: familial thrombophilia (familial protein S deficiency), amplified by ERT-mediated thrombophilia, caused not only the patient's osteonecrosis, but also the hyperglucagonemia, diarrhea, and abdominal pain by virtue of mesenteric artery or vein thrombosis and ischemia. Mesenteric artery or vein imaging were not carried out because the patient's hyperglucagonemia, diarrhea, and abdominal pain disappeared after stopping ERT, removing the clinical indication for invasive angiography. Hence, we cannot provide anatomic proof for the postulate that the hyperglucagonemia, diarrhea, and abdominal pain were caused by mesenteric artery or vein thrombosis and intestinal and pancreatic ischemia.

The relationship between osteonecrosis, thrombophilia and hypofibrinolysis has been recently examined [13, 17, 18, 20, 21, 22, 23]. In a study of avascular necrosis of the hip, 74% of all patients had one or more primary coagulation disorders [20]. It has been suggested that coagulation disorders leading to venous thrombosis may result in interosseous venous hypertension. The interosseous hypertension causes bone cell ischemia and gradually leads to bone cell death [13, 17, 18, 20, 21, 22, 23]. ERT is known to reduce free protein S [14, 15] and osteonecrosis has been shown to result from thrombophilic ERT given to patients with underlying thrombophilic protein S deficiency [17, 18]. We suspect that these mechanisms were present in our patient with protein S deficiency and on ERT. In her case, the enhanced familial hypercoagulability resulted in osteonecrosis of the hip and ultimately to total hip replacement. Similar sequences have previously been reported for osteonecrosis of the jaw [17, 18].

In the present case, the patient's glucagon levels and glucagon-mediated symptoms were controlled only after starting daily octreotide

therapy. Hence, glucagon levels would be expected to rise and symptoms to worsen after octreotide was stopped. However, this did not occur. The concurrent cessation of the octreotide and ERT led to no change in the patient's previously pharmaceutically normalized levels of glucagon. Moreover, glucagon levels remained normal over 30 months of follow-up after cessation of octreotide and ERT. We speculate that this occurred because the thrombotic interaction of ERT and familial protein S deficiency was responsible for the original elevation of the patient's glucagon, her abdominal pain, and her intractable diarrhea. Several cases of protein S deficiency and mesenteric venous thrombosis have been described with ischemic and/or necrotic bowel [9, 26, 27, 28, 29]. In familial protein S deficiency, venous thrombosis may occur in as many as 55% of patients, with recurrent events in 77% [9]. Pancreatic ischemia studies have revealed elevated amylase and lipase levels but not hyperglucagonemia [30]. We speculate that thrombophilic familial protein S deficiency was amplified by ERT [14] and produced mesenteric vein thrombosis with resultant pancreatic ischemia. We postulate further that pancreatic ischemia led to oversecretion of glucagon by the pancreas. We suspect that the patient's chronic abdominal pain, even while on octreotide, reflected mesenteric ischemia. Finally, it is likely that the patient's intractable diarrhea was a further manifestation of mesenteric ischemia, independent of the hyperglucagonemia, given its persistence beyond normalization of glucagon levels, until ERT was discontinued.

Heritable coagulation disorders, particularly when augmented by exogenous thrombophilic factors like ERT, may speculatively, be pathoetiological for mesenteric-pancreatic ischemia and hyperglucagonemia.

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Key words Glucagon; Splanchnic Circulation; Thrombophilia

Abbreviations EGD: esophagogastro-duodenoscopy; ERT: estrogen replacement therapy; Lp(a): lipoprotein (a); MTHFR: methylenetetrahydrofolate reductase; PAI-1: plasminogen activator inhibitor-1 gene; PAI-Fx: plasminogen activator inhibitor activity

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